# NONVIRAL TRANSFER OF THE GENE ENCODING COAGULATION FACTOR VIII IN PATIENTS WITH SEVERE HEMOPHILIA A

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## ABSTRACT

*Background* We tested the safety of a nonviral somatic-cell gene-therapy system in patients with severe hemophilia A.

*Methods* An open-label, phase 1 trial was conducted in six patients with severe hemophilia A. Dermal fibroblasts obtained from each patient by skin biopsy were grown in culture and transfected with a plasmid containing sequences of the gene that encodes factor VIII. Cells that produced factor VIII were selected, cloned, and propagated in vitro. The cloned cells were then harvested and administered to the patients by laparoscopic injection into the omentum. The patients were followed for 12 months after the implantation of the genetically altered cells. An interim analysis was performed.

*Results* There were no serious adverse events related to the use of factor VIII–producing fibroblasts or the implantation procedure. No long-term complications developed, and no inhibitors of factor VIII were detected. In four of the six patients, plasma levels of factor VIII activity rose above the levels observed before the procedure. The increase in factor VIII activity coincided with a decrease in bleeding, a reduction in the use of exogenous factor VIII, or both. In the patient with the highest level of factor VIII activity, the clinical changes lasted approximately 10 months.

*Conclusions* Implantation of genetically altered fibroblasts that produce factor VIII is safe and well tolerated. This form of gene therapy is feasible in patients with severe hemophilia A. (N Engl J Med 2001; 344:1735-42.)



HEMOPHILIA A, an X-linked hemorrhagic disorder due to mutations in the gene that encodes factor VIII, affects 1 in 5000 males.[1] Approximately 60 percent of patients with a mutant gene for factor VIII have severe hemophilia (in which the level of factor VIII activity is less than 1.0 percent of normal), whereas the remainder have moderate or mild hemophilia (factor VIII activity, 1.0 to 5.0 percent of normal or more than 5.0 percent of normal, respectively).[1] In severe hemophilia, spontaneous bleeding into joints, soft tissues, and vital organs is frequent, whereas in mild hemophilia, bleeding usually occurs only after trauma or surgery.[2]

Replacement with intravenously administered concentrates of factor VIII controls bleeding in patients with hemophilia A. In patients with severe disease, spontaneous hemorrhage and progression of arthropathy can be prevented by prophylactic administration of exogenous factor VIII to maintain factor VIII activity levels above 1.0 percent of normal.[3] Prophylaxis with factor VIII is costly,[1] however, and does not guarantee the prevention of hemorrhage.[4] Despite considerable improvements in the safety of plasma-derived and recombinant factor VIII concentrates, concern remains about contamination with infectious prions[5,6] and small nonenveloped viruses, such as hepatitis A virus[7,8] and parvovirus.[9-11] Hemophilia A is a suitable candidate disease for gene therapy[12] for several reasons: factor VIII production is not regulated in response to bleeding; the broad therapeutic index of factor VIII minimizes the risk of overdoses; delivery of factor VIII into the bloodstream does not require expression of the gene by a specific organ; and even low levels of the protein can be beneficial.

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We have developed a nonviral gene-delivery system termed "transkaryotic implantation." It entails the isolation of somatic cells from a patient, the stable introduction of a therapeutic gene into these cells, the isolation and clonal propagation of a single engineered cell, and the implantation of the clonal cells into the patient.[13-15] Mice that received fibroblast implants carrying DNA sequences of the human factor VIII gene produced the human protein; the procedure was safe and resulted in factor VIII activity levels that exceeded 5.0 percent of normal — a level that would be considered therapeutic in patients with severe hemophilia A — for more than one year (unpublished data). These results led us to study the safety of the transkaryotic-implantation system in patients with severe hemophilia A.

## METHODS

### Patients

The study was designed as a single-institution, open-label, phase 1 trial in which autologous fibroblasts that produced human factor VIII were administered to patients with severe hemophilia A. Patients were eligible to be included in the study if they had hemophilia A with a level of factor VIII activity below 2.0 percent of normal; were at least 15 years old; had received at least 50 days of factor VIII therapy before study entry; had six or more bleeding episodes per year; had normal factor VIII clearance; had a hemoglobin level above 12 g per deciliter (7.4 mmol per liter); and had a platelet count above 100,000 per cubic millimeter. Patients with any of the following characteristics were excluded: the presence of a factor VIII inhibitor, as determined by the Bethesda inhibitor assay (which detects antibodies that neutralize factor VIII activity) at the time of enrollment; a history of inducible factor VIII inhibitor; an anticipated requirement for fixed-dose prophylaxis with factor VIII infusions during the study; a history of opportunistic infection or cancer related to the acquired immunodeficiency syndrome; use of investigational therapy for hemophilia within 30 days before enrollment in the study; or clinical evidence of abdominal adhesions or portal hypertension (which would increase the risk associated with the laparoscopic procedure).

The clinical protocol and the informed-consent document were approved by the Center for Biologics Evaluation and Research of the Food and Drug Administration and by the Office of Biotechnology Activities of the National Institutes of Health. Local review and approval were obtained from the Beth Israel Deaconess Medical Center institutional review board, the Harvard University Biosafety Committee, and the Harvard University Human Gene Therapy Advisory Committee. Permission to treat up to 12 patients and to monitor them for up to two years was given. All the patients provided written informed consent.

Figure 1 shows the steps involved in the study. First, the patients underwent a clinical evaluation in which a complete medical history was obtained and a physical examination, blood and urine tests, electrocardiography, and chest radiography were performed. Patients received diary forms for use at home to record and describe all bleeding episodes and all infusions of factor VIII during the study. The date, time, and site of bleeding were recorded for each episode, as were the number of units of factor VIII administered with each infusion. The reason for factor VIII infusion was assigned to one of the following categories: spontaneous bleeding, injury-related bleeding, prophylaxis, and other reasons (including use for the skin biopsy, the laparoscopy, and other procedures).

### Skin Biopsy

A full-thickness, elliptical skin-biopsy specimen (1.6 by 0.4 cm) was obtained from the medial surface of the upper arm after the patient received local anesthesia. Dermal fibroblasts were isolated for cell culture and subsequent plasmid transfection. All the patients received an infusion of factor VIII before the biopsy to increase their plasma factor VIII activity level to 100 percent of normal. Factor VIII was administered for an additional three or four days to maintain normal hemostasis.

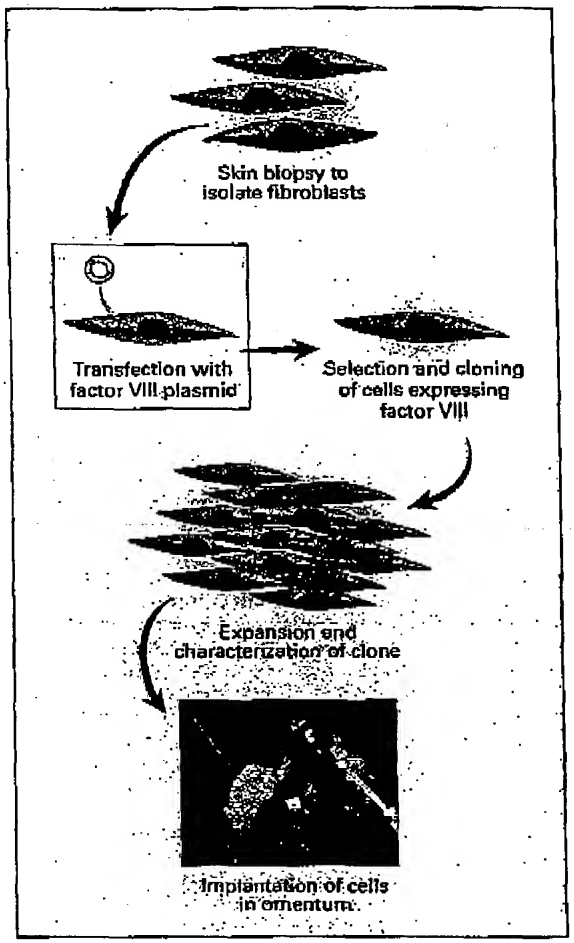


**Figure 1. Steps in the Human Factor VIII Gene-Transfer Procedure.**
The fibroblasts from the skin-biopsy specimen were transfected with a plasmid containing the gene encoding human factor VIII from which the B domain had been deleted.

### Laparoscopy

Approximately seven weeks after the skin biopsy, the patients returned to the hospital for another clinical assessment, which was performed the day before cell implantation. Factor VIII was infused just before surgery to increase plasma factor VIII activity levels to approximately 100 percent of normal, and the infusions were continued two or three times daily for one week after surgery. The laparoscopic procedure was performed while the patients were under general anesthesia. A 10-mm trocar was inserted into the peritoneal

cavity through an infraumbilical incision with the use of an open technique. After carbon dioxide insufflation, a laparoscope was introduced at this site. Under direct vision, a single, 5-mm operating trocar was inserted into the midabdomen, and through this trocar, a laparoscopic grasper was used to manipulate the greater omentum. A 9-cm (3.5-in.), 20-gauge spinal needle was inserted directly through the abdominal wall under direct vision, and approximately 0.5 ml of the cell suspension was injected through the needle into the greater omentum at each of several sites. After surgery, the patients were observed overnight in the clinical research center. All six patients were discharged home on the first postoperative day.

Clinical assessments at the patients' homes were performed daily by the study nurse on days 2 through 6 after surgery. The patients were scheduled to undergo clinical assessments at the hospital during weeks 1, 2, 3, 4, 6, 8, 12, and 18 and months 6, 9, and 12 after cell implantation, with additional monitoring through year 2.

### Preparation of Autologous Fibroblasts Expressing Factor VIII

A plasmid that contained the gene encoding human factor VIII from which the B domain had been deleted and that contained the human fibronectin promoter was introduced by electroporation into dermal fibroblasts that had been isolated from the skin-biopsy specimen. The B-domain coding sequence was not incorporated into the plasmid, because it is not required for the coagulant activity of factor VIII or the interaction of factor VIII with von Willebrand factor[16,17]; moreover, the presence of the B domain reduces expression of factor VIII in transfected mammalian cells. Fibronectin, an extracellular-matrix protein, is expressed in fibroblasts; its promoter efficiently directs the transcription of B-domain–deleted factor VIII in primary human dermal fibroblasts.

Stably transfected fibroblast clones that had incorporated the plasmid containing B-domain–deleted factor VIII were selected in G418-containing medium, isolated, expanded in nonselective medium, and characterized. Characterization included measurement of factor VIII expression, assessment of cell-growth properties (including soft-agar tumorigenicity assay in vitro), microbial safety, and Southern blotting. Cells from the clone that was designated for implantation were harvested the day before implantation, extensively washed, and introduced into a syringe. Either 100 million or 400 million cloned cells were then administered. During preparation, the fibroblast cultures were aseptically processed in class 100 conditions.[18]

### Factor VIII Assays

Standard one-stage factor VIII clotting-activity assays and Bethesda inhibitor assays based on measurement of the activated partial-thromboplastin time were performed (Automated APTT reagent, Organon Teknika) on an MDA instrument with the use of a standard curve prepared with a normal human pooled plasma calibration standard (Precision Biologic), which was calibrated against a World Health Organization standard. Abnormal controls (Dade and Behring) and normal controls (Precision Biologic), both derived from human plasma, were used to validate the assays. Serum samples from the patients were analyzed for antibodies against factor VIII with two specific enzyme-linked immunosorbent assays. Full-length human factor VIII derived from Chinese-hamster-ovary cells (Recombinate, Hyland–Baxter) or human plasma–derived factor VIII (Hemofil-M, Hyland–Baxter) was used to coat the wells of microtiter plates before the addition of patients' serum. The enzyme-linked immunosorbent assay was developed with a horseradish peroxidase–conjugated goat antihuman antibody. The assay was validated with the use of normal human plasma and abnormal human plasma containing various amounts of factor VIII antibodies (George King Bio-Medical). All specialized blood-coagulation testing was performed at Esoterix Coagulation Laboratories with use of frozen plasma or serum samples that were collected from the patients at all scheduled visits; however, at month 9 only factor VIII activity was tested, and only in Patients 5 and 6.

## RESULTS

### Characteristics of the Patients and Factor VIII Production by the Implanted Fibroblasts

Table 1 shows the characteristics of the six patients. As shown in Table 2, Patients 1, 2, and 3 each received 100 million cells, and Patients 4, 5, and 6 each received 400 million cells. The total factor VIII production of each of the clones that was implanted is also shown.

### Safety

The skin biopsy was well tolerated by all six patients, and there were no episodes of bleeding or infectious

TABLE 1. CHARACTERISTICS OF THE SIX PATIENTS.*

| CHARACTERISTIC | VALUE |
|---|---|
| Age (yr) | |
| Mean | 46 |
| Range | 20–72 |
| Weight (kg) | |
| Mean | 70 |
| Range | 50–91 |
| Pretreatment factor VIII activity <0.8% of normal (no. of patients) | 6 |
| Viral exposure (no. of patients)† | |
| Human immunodeficiency virus | 4 |
| Hepatitis A virus | 5 |
| Hepatitis B virus | 5 |
| Hepatitis C virus | 6 |

*All six patients were men.

†Viral exposure was determined at the time of enrollment by testing for the presence of antibodies to the viruses listed.

TABLE 2. TOTAL FACTOR VIII PRODUCTION BY IMPLANTED AUTOLOGOUS FIBROBLASTS.*

| PATIENT NO. | FACTOR VIII PRODUCTION BY HARVESTED CELLS† | NO. OF CELLS IMPLANTED | TOTAL FACTOR VIII PRODUCTION BY IMPLANTED CELLS‡ |
|---|---|---|---|
| | IU/10⁶ cells/day | | IU/kg/day |
| 1 | 0.8 | 100×10⁶ | 1.3 |
| 2 | 4.9 | 100×10⁶ | 5.4 |
| 3 | 1.9 | 100×10⁶ | 3.8 |
| 4 | 1.8 | 400×10⁶ | 10.4 |
| 5 | 1.6 | 400×10⁶ | 8.4 |
| 6 | 6.7 | 400×10⁶ | 36.0 |

*The conditioned medium of each fibroblast clone was replaced with fresh medium 24 hours before it was assayed for factor VIII expression levels by a human factor VIII enzyme-linked immunosorbent assay.

†The production of factor VIII at the time of cell harvest, before implantation, is shown.

‡The total factor VIII production of each implanted clone is shown, normalized for the weight of each patient.

complications. Laparoscopic implantation of the fibroblasts was also well tolerated. The mean (±SD) duration of surgery was 65±7 minutes. The mean time required for the injection of the fibroblasts was 15±3 minutes. There were no complications related to anesthesia. After the procedure, all six patients had minor abdominal discomfort at the incision site. Other adverse events during the postoperative period were pain referred to the shoulder in five patients, abdominal ecchymoses in two, and low-grade postoperative fever (which did not require treatment) in one. There were no long-term complications associated with surgery.

All the Bethesda inhibitor assays and enzyme-linked immunosorbent assays for antibodies against factor VIII were negative. Preliminary assays for cytotoxic T lymphocytes at selected times through month 12 in Patients 1, 2, and 3 and through month 6 in Patients 4, 5, and 6, in which the patients' lymphocytes were used as the effector cells and transfected autologous fibroblasts expressing B domain–deleted factor VIII were used as the target, were negative; this finding indicates that there was no cellular immune response to the cultured transfected fibroblasts.

One patient with a long-standing history of recurrent paroxysmal atrial tachycardia had two episodes of tachycardia, 7.5 months and 12 months after the implantation procedure. These two serious adverse events were classified as unrelated to the use of the genetically modified fibroblasts or the implantation. There were no other serious adverse events. All six patients had adverse events, most of which consisted of bleeding episodes or pain associated with bleeding. No clinically significant laboratory abnormalities related to the gene therapy were detected.

#### Clinical Effects

In addition to evaluating the safety of the use of the genetically engineered fibroblasts and of the surgical procedures (skin biopsy and laparoscopy), we appraised several subjective and objective measures of efficacy: bleeding events related to hemophilia, use of exogenous factor VIII for treatment before and during the study, and factor VIII activity levels. Figure 2 shows bleeding events and the use of exogenous factor VIII in Patients 1, 3, and 6, all of whom maintained diaries of bleeding and factor VIII use before participation in the study. Patients 2, 4, and 5 did not keep such diaries before entering the study.

In Patient 1, the frequency of bleeding and the amount of exogenous factor VIII used did not change after implantation of the transfected fibroblasts. This patient received the implant with the lowest level of factor VIII production in vitro of any of the implants (Table 2), and no clinically important increase in factor VIII activity was detected in his plasma after therapy (Table 3). Beginning 10 weeks after the implantation of transfected fibroblasts, Patient 3 had no bleeding, and he did not use factor VIII for the treatment of new bleeding for the next 3 months. At week 18, nearly eight weeks after the most recent infusion of factor VIII, his plasma factor VIII activity had increased from less than 0.4 percent of normal to 2.0 percent of normal (Table 3). There were injury-related bleeding episodes, but he reported no spontaneous bleeding. There were several prolonged intervals without bleeding or infusions of factor VIII (Fig. 2). Additional measurements of factor VIII activity showed an activity level of 1.0 percent of normal at 6 months and a decline to less than 0.5 percent at 12 months.

Patient 6 received the fibroblast clone that produced the largest amount of factor VIII per day in vitro of any of the clones (Table 2). A decreased frequency of bleeding became evident one month after the transfected fibroblasts were implanted (Fig. 2). During periods when there was no bleeding or use of exogenous factor VIII, numerous measurements of factor VIII activity showed a persistent increase in activity to a level above that observed at the initial evaluation (Table 3). Like Patient 3, he reported no spontaneous bleeding for approximately 10 months after the implantation (Fig. 2). However, there was intermittent injury-related hemorrhage. The frequency of bleeding in this patient increased and spontaneous bleeding recurred during month 12, at which time his level of factor VIII activity had declined to less than 0.5 percent of normal (Table 3).

Table 4 shows the average monthly use of factor VIII before and after cell implantation in all six patients. Treatment with infusions of exogenous factor VIII before enrollment in the study was documented by the patients' diaries (Patients 1, 3, and 6) or by pharmacy records (Patients 2, 4, and 5). Use of exogenous factor VIII by Patients 1 and 2 was unchanged after treatment. Use by Patients 3, 4, and 5 was moderately decreased. In Patient 6 there was a considerable reduction in the use of exogenous factor VIII after gene therapy; in the 20 months before therapy, his total use of factor VIII each month ranged from 12,339 to 30,621 U, whereas his use ranged from 2040 to 7098 U per month at months 4, 6, 7, 8, and 10 after gene therapy (Table 4).

A standard one-stage assay of factor VIII activity was used to detect changes in factor VIII activity over time (Table 3). Levels of factor VIII activity above pretreatment levels were repeatedly found after treatment in Patients 3, 4, 5, and 6. Patient 3 had a factor VIII activity level of 2.0 percent of normal at week 18 (eight weeks after his most recent factor VIII infusion). It was 1.0 percent of normal at month 6, nine days after a single infusion of factor VIII. These elevated levels were not the result of the use of exogenous factor VIII, because factor VIII activity was undetectable at other points closer in time to a preceding infusion (weeks 2, 6, 8, and 12). Patient 4 had factor VIII activity levels of 0.8 percent and 0.6 percent of normal at weeks 4 and 6, respectively. Although both

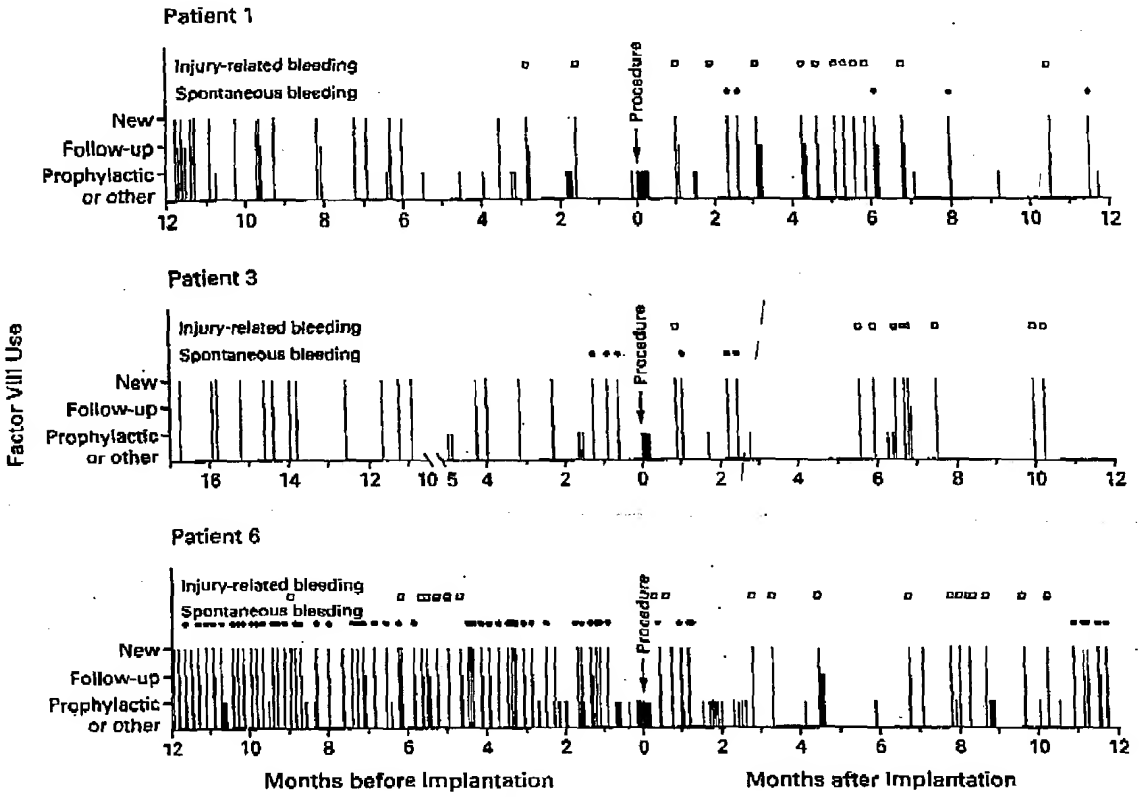


**Figure 2. Bleeding Events and Use of Exogenous Factor VIII in Three of the Six Patients.**

Bleeding events and use of exogenous factor VIII during the 12 months before and after the cell-implantation procedure (time 0) are shown. Each infusion of factor VIII is shown as a vertical line. The tallest lines correspond to infusions at the time of a new bleeding event. After enrollment in the study each of these new bleeding episodes was classified as injury related (open squares) or spontaneous (solid circles). Lines of intermediate height represent follow-up infusions to complete the treatment of a previous bleeding episode, and the shortest lines represent infusions for prophylaxis or other reasons, such as the surgical procedures in the study. In the chart for Patient 3, pretreatment data over a 5-month period (between 10 months and 5 months before the implantation) are omitted; during this period the patient used fixed-dose factor VIII as secondary prophylaxis, and neither the frequency of bleeding nor the use of factor VIII was representative of that at other times before entry into the study. For Patients 1 and 3, bleeding episodes were recorded but not classified until enrollment in the study 3.3 and 1.7 months, respectively, before the implantation procedure. For Patient 6, episodes that occurred 11.9, 11.8, 11.5, 7.7, and 2.3 months before and 1.0 and 7.1 months after the implantation procedure were inadvertently not classified. Monthly intervals are 30 days.

of these levels were above his initial level before the implantation (less than 0.4 percent of normal), it is unclear whether these small changes are clinically significant. Nevertheless, the appearance of measurable factor VIII coincided with decreased use of exogenous factor VIII. In Patient 5, several measurements of factor VIII activity between week 4 and month 6 after the implantation were above his initial level (which was undetectable). Although the change in the use of factor VIII was small in this patient, there was a general correlation between decreased use of factor VIII through month 10 and the duration of the elevation in factor VIII activity. Patient 6 had the highest measurable level of activity after gene therapy; at week 12, the activity was 4.0 percent of normal. At months 9 and 12, the activity in this patient had decreased to less than 0.5 percent of normal, and spontaneous bleeding recurred.

## DISCUSSION

We studied six patients with severe hemophilia A who received autologous fibroblasts that carried the gene that encodes factor VIII. The implantation procedure was safe, and the cells have been well tolerated,

TABLE 3. LEVELS OF FACTOR VIII ACTIVITY.*

| TIME POINT | PATIENT | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| | % of normal level | | | | | |
| Initial evaluation | <0.8 | <0.8 | <0.8 | <0.4 | 0 | 0.5 |
| Day before cell implantation | <0.8 (3 to <4 days) | <0.8 | <0.4 | <0.4 | <0.4 (3 to <4 days) | 4.0 (1 to <2 days) |
| Week 2 | <0.8 | <0.8 | <0.4 | <0.8 | 3.0 (1 to <2 days) | 102 (<1 day) |
| Week 3 | <0.8 | <0.4 | <0.8 | <0.4 | 2.0 (3 to <4 days) | 2.0† |
| Week 4 | <0.8 | <0.4 | 19.0 (<1 day) | 0.8† | 1.0† | 3.0† |
| Week 6 | <0.8 | 0.8 (3 to <4 days) | <0.4 | 0.6† | 18.0 (<1 day) | 1.0† |
| Week 8 | <0.8 | <0.4 | <0.4 | <0.5 | 1.0† | 5.0 (1 to <2 days) |
| Week 12 | <0.8 | <0.4 | <0.4 | <0.4 | 1.0† | 4.0† |
| Week 18 | <0.4 | <0.4 | 2.0† | <0.5 | 0.5† | 1.0† |
| Month 6 | <0.4 | 0.7 | 1.0† | <0.5 | 1.0† | 2.0† |
| Month 9 | — | — | — | — | 1.0 (1 to <2 days) | <0.5 |
| Month 12 | 0.5 | <0.5 | <0.5 | 0.5† | <0.5 | <0.5 |

*Some measurements of factor VIII activity levels were most likely influenced by previous infusions of exogenous factor VIII. The interval between measurement and the most recent infusion of factor VIII is given in parentheses. Otherwise, the factor VIII activity level was determined at least five days after a previous factor VIII infusion. Factor VIII activity levels were measured at scheduled visits: weeks 2 and 3 (±1 day); weeks 4 and 6 (±2 days); weeks 8 and 12 (±4 days); and week 18 and months 6, 9, and 12 (±2 weeks). Dashes indicate that measurement of factor VIII activity was not performed.

†The factor VIII activity level is considered elevated above levels measured before implantation.

a finding that has continued in all patients during a second year of monitoring. In no patient did evidence of factor VIII inhibitor appear at any time during the study. This is important, because inhibitors have the potential to compromise the effectiveness of standard factor VIII–replacement therapy.[19]

The potential for clinical efficacy of this type of gene therapy is suggested by the increased levels of factor VIII activity in four of the six patients. Before they entered the study, the factor VIII activity levels in all six patients were less than 0.8 percent of normal — a fact that made it possible to detect relatively small increases in factor VIII in plasma after the implantation of the genetically engineered fibroblasts. There was a general correlation between these increases and clinical improvement, such as a decreased frequency of spontaneous-bleeding episodes or decreased use of exogenous factor VIII.

At the relatively low levels of factor VIII activity we observed, several factors may have influenced subjective measures of the clinical efficacy of the gene-therapy procedure we tested. One of these is the presence or absence of active joint disease: treatment of severe joint disease requires persistent, relatively high levels of factor VIII activity. The patients' investment in the therapeutic outcome of the experimental treatment may also have influenced subjective measures of efficacy that suggested therapeutic benefit. However, the correlation between objective and subjective signs of improvement indicates that a prolonged placebo effect was unlikely.

The minimal levels of factor VIII that are required to protect against spontaneous or post-traumatic bleeding are unknown. Protection against bleeding related to trauma or surgery may require high factor VIII levels, in the range of 30 to 100 percent of normal, depending on the nature of the injury.[20] In contrast, the clinical features that distinguish between severe and moderate hemophilia suggest that factor VIII activity levels as low as 1.0 to 2.0 percent of normal can protect against spontaneous bleeding. A recent study of prophylactic treatment in patients with severe hemophilia indicated that even levels below 1.0 percent of normal may be sufficient.[21] For these reasons, the clinical responses we observed in our patients, who had low plasma levels of factor VIII, are encouraging.

The nonviral ex vivo gene-therapy system we used has several potential advantages over gene therapy based on viral vectors. Patients received a homogeneous clonal population of cells containing a single genetic modification, thereby minimizing the risk of insertional mutagenesis that might occur with viral vectors. In addition, viral vectors can evoke immune responses that may attenuate the effectiveness of sub-

TABLE 4. MONTHLY USE OF EXOGENOUS FACTOR VIII.*

| PATIENT NO. | REASON FOR FACTOR VIII TREATMENT | BEFORE CELL IMPLANTATION† | MONTHS AFTER CELL IMPLANTATION | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| | | | IU of factor VIII | | | | | | | | | | | |
| 1 | Spontaneous bleeding | | 0 | 0 | 5,153 | 0 | 0 | 0 | 11,766 | 2,220 | 2,220 | 0 | 0 | 4,480 |
| | Injury-related bleeding | | 0 | 4,100 | 0 | 10,024 | 15,326 | 8,320 | 6,426 | 0 | 0 | 0 | 2,220 | 0 |
| | Prophylaxis or other | | 14,431 | 4,160 | 0 | 0 | 0 | 0 | 0 | 2,220 | 0 | 2,220 | 0 | 2,240 |
| | Total | 5,904±4,617 | 14,431 | 8,260 | 5,153 | 10,024 | 15,326 | 8,320 | 18,192 | 4,440 | 2,220 | 2,220 | 2,220 | 6,720 |
| 2 | Spontaneous bleeding | | 2,000 | 2,000 | 0 | 0 | 2,060 | 2,060 | 6,140 | 6,180 | 0 | 0 | 4,120 | 2,060 |
| | Injury-related bleeding | | 0 | 0 | 4,000 | 4,120 | 2,020 | 0 | 0 | 0 | 1,010 | 0 | 0 | 2,020 |
| | Prophylaxis or other | | 28,860 | 0 | 0 | 2,020 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Total | 4,798 | 30,860 | 2,000 | 4,000 | 6,140 | 4,080 | 2,060 | 6,140 | 6,180 | 1,010 | 0 | 4,120 | 4,080 |
| 3 | Spontaneous bleeding | | 0 | 2,080 | 2,625 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Injury-related bleeding | | 2,865 | 0 | 0 | 0 | 0 | 4,300 | 6,550 | 2,200 | 0 | 0 | 3,825 | 0 |
| | Prophylaxis or other | | 15,283 | 1,040 | 1,050 | 0 | 0 | 0 | 2,200 | 0 | 0 | 0 | 0 | 0 |
| | Total | 3,726±1,799 | 18,148 | 3,120 | 3,675 | 0 | 0 | 4,300 | 8,750 | 2,200 | 0 | 0 | 3,825 | 0 |
| 4 | Spontaneous bleeding | | 3,264 | 2,736 | 5,510 | 7,996 | 3,831 | 3,360 | 8,768 | 2,992 | 20,218 | 10,282 | 24,542 | 17,535 |
| | Injury-related bleeding | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Prophylaxis or other | | 15,833 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5,600 | 2,510 | 0 | 2,721 |
| | Total | 16,303 | 19,097 | 2,736 | 5,510 | 7,996 | 3,831 | 3,360 | 8,768 | 2,992 | 25,818 | 12,792 | 24,542 | 20,256 |
| 5 | Spontaneous bleeding | | 5,250 | 6,940 | 11,068 | 13,024 | 8,320 | 10,175 | 4,625 | 8,495 | 9,090 | 10,100 | 13,100 | 20,928 |
| | Injury-related bleeding | | 2,100 | 2,100 | 1,956 | 10,980 | 2,080 | 0 | 2,775 | 1,850 | 0 | 0 | 0 | 2,160 |
| | Prophylaxis or other | | 28,450 | 0 | 1,956 | 0 | 0 | 0 | 0 | 925 | 3,030 | 1,010 | 2,988 | 1,992 |
| | Total | 14,630 | 35,800 | 9,040 | 14,980 | 24,004 | 10,400 | 10,175 | 7,400 | 11,270 | 12,120 | 11,110 | 16,088 | 25,080 |
| 6 | Spontaneous bleeding | | 9,031 | 4,240 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2,196 | 23,564 |
| | Injury-related bleeding | | 4,925 | 0 | 2,316 | 2,040 | 22,340 | 0 | 5,298 | 3,532 | 6,242 | 5,490 | 2,196 | 0 |
| | Prophylaxis or other | | 23,019 | 15,040 | 9,960 | 0 | 1,766 | 4,420 | 0 | 3,566 | 18,174 | 0 | 5,728 | 0 |
| | Total | 19,052±5,133 | 36,975 | 19,280 | 12,276 | 2,040 | 24,106 | 4,420 | 5,298 | 7,098 | 24,416 | 5,490 | 10,120 | 23,564 |

*The patients recorded their use of factor VIII on diary forms and categorized each infusion according to the reason for administration. When a bleeding episode was treated with more than a single infusion of factor VIII, all the follow-up infusions were classified in the same category as the initial infusion. The category "prophylaxis or other" includes the use of factor VIII for surgical procedures such as the skin biopsy, the cell-implantation procedure, or other procedures, such as dental work.

†Data were available for the following intervals before treatment: Patient 1, 13 months; Patient 2, 14 months; Patient 3, 28 months (except the period from 10 months through 5 months before implantation, when he took a fixed-dose factor VIII as secondary prophylaxis); Patient 4, 14 months; Patient 5, 24 months; and Patient 6, 20 months. All monthly intervals were 30 days. Plus–minus values are means ±SD and represent monthly averages for data taken from patient diaries. When average monthly use was determined from pharmacy records, the SD is omitted.

sequent treatments.[22] Viral vectors may become infectious by mutation or other mechanisms or may modify the patient's germ line, but these risks are obviated with the use of the nonviral approach. Despite these advantages, our system has noteworthy disadvantages. The implantation procedure is moderately invasive, and the factor VIII–producing autologous fibroblasts must be prepared individually for each patient. Fortunately, there is no technological obstacle to the production of such patient-specific fibroblasts, so the procedure could be feasible if the therapeutic benefit of this approach can be proved in subsequent studies.

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Drs. Treco and Selden are employees of and own stock in Transkaryotic Therapies.

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## APPENDIX

Other members of the Factor VIII Transkaryotic Therapy Study Group were as follows (asterisks indicate a financial interest in Transkaryotic Therapies): J.D. Levine, J. Proper, and B. Furie* (Beth Israel Deaconess Medical Center and Harvard Medical School, Boston) and V.A. Roman,* Z.M. Sabine,* C.W. Phillips,* A.M. Zuliani,* N.A. Savioli,* D. Fisher,* J. Harrington,* M. Borowski,* M.W. Heartlein,* J.C. Lamsa,* E.M. Morrel,* and K.C. Gunter* (Transkaryotic Therapies, Cambridge, Mass.)

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